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Published in:
Journal of Experimental Medicine

DOI:
[10.1084/jem.153.1.30](https://doi.org/10.1084/jem.153.1.30)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1981

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Poppema, S., Bhan, A. K., Reinherz, E. L., McCluskey, R. T., & Schlossman, S. F. (1981). Distribution of T cell subsets in human lymph nodes. *Journal of Experimental Medicine*, 153(1), 30-41.
<https://doi.org/10.1084/jem.153.1.30>

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DISTRIBUTION OF T CELL SUBSETS IN HUMAN LYMPH NODES*

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Multiple interactions between T and B lymphocytes are required for generation of the immune response. In large part, these interactions appear to require direct cell-cell contact. Therefore, determination of the precise location of T and B cells within lymphoid structures is of considerable importance. In laboratory animals, approaches utilized to define the anatomic relationship of lymphocyte subpopulations have included determination of the effects of thymectomy on lymphoid architecture (1), study by autoradiography of localization of intravenously injected radiolabeled T and B cell populations (2-4), and immunofluorescence studies of lymphoid tissue employing specific heteroantisera (5). Such studies have provided evidence for a general anatomic compartmentalization of lymphocyte subpopulations. Thus, within lymph nodes, B cells predominate in follicular areas, whereas T cells comprise the predominant cell type in the paracortical areas. In man, immunofluorescence studies of normal lymphoid structures, as well as histologic examination of lymph node tissues from patients with T or B cell deficiency states, have shown similar findings (6-8). These studies have not permitted determination of the location of T cell subsets.

Recently, a series of monoclonal antibodies reactive with human thymocyte and peripheral T cell antigens have been developed (9-14). These antibodies allow for the precise characterization of lymphocytes of T lineage. For example, anti-T1 and anti-T3 antibodies define the entire population of mature peripheral T cells, whereas anti-T4 and anti-T5/T8 are reactive selectively with the inducer and suppressor/cytotoxic subsets, respectively. In addition, a monoclonal antibody termed anti-T6 defines a differentiation antigen expressed on the common thymocyte population but lacking on peripheral T cells. In a prior report, we have employed these antibody probes to localize T cell-associated antigens in the human thymus by immunofluorescence and immunoperoxidase techniques (15). The present study makes use of these monoclonal antibodies, as well as antibodies directed at human IgM and the common framework portion of Ia antigen, to determine the in situ anatomic relationship of immunoregulatory T cell subsets to the B cell-dependent areas in human lymph nodes.

Materials and Methods

Source of Lymph Nodes. The lymph nodes used for the study were obtained from surgical procedures done for diagnostic or therapeutic purposes at the Massachusetts General Hospital,

* Supported by U. S. Public Health Service grants AI 12069, CA 19589, CA 06516, and HL-18646.

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TABLE I
Source and Appearance of Lymph Nodes

Case number	Age	Sex	Location of lymph node	Histologic features
1	31 ^{yr}	M	Cervical	Follicular and diffuse cortical hyperplasia with melanin-containing macrophages, consistent with dermatopathic lymphadenitis
2	62	M	Inguinal	Follicular hyperplasia with granulomatous reaction and rare microabscesses; changes suggestive of infectious etiology
3	29	M	Axillary	Follicular hyperplasia
4	30	F	Postauricular	Follicular hyperplasia with epithelioid cell collections, consistent with toxoplasma lymphadenitis
5	68	F	Axillary (radical mastectomy for carcinoma of the breast)	Sinus histiocytosis

Boston, Mass. The specimens were snap frozen in OCT compound and stored at -70°C . Lymph nodes from five patients were studied. The lymph nodes showed a variety of histological reactions, as listed in Table I.

Description of Antibodies. A series of monoclonal antibodies that are reactive with T lymphocyte surface antigens were employed. The antibodies were obtained in the form of ascitic fluid from mice injected with hybridoma cells. Control ascitic fluid was obtained from animals injected with nonsecreting hybridoma cells. The methods of production and characterization of the antibodies have been described earlier (9-14). In brief, anti-T1 and anti-T3 antibodies react with 100% of peripheral T cells and 10% of thymocytes, whereas anti-T6 antibody reacts with 70% of thymocytes and not with peripheral T cells. Anti-T4 antibody, which defines helper/inducer T cells, reacts with approximately 55% of peripheral T cells. In contrast, anti-T5 and anti-T8 antibodies, which define suppressor/cytotoxic T cells, react with ~20-30% of peripheral T cells.

In addition, an anti-Ia monoclonal antibody (anti-I1),¹ reactive with a nonpolymorphic region of human Ia-like antigens (16) and a rabbit anti-human IgM antiserum (Dakopatts A/S, Copenhagen, Denmark) were used.

Immunoperoxidase Procedures. A four-step immunoperoxidase technique using peroxidase-antiperoxidase complexes as the final step was applied; earlier experiments indicated a higher sensitivity with this method than with immunofluorescence or conjugated immunoperoxidase techniques (15, 17). 5- μm thick frozen sections were stored at -20°C and air-dried with a ventilator for 20 min before use. The sections were fixed in acetone for 10 min at room temperature and washed in phosphate-buffered saline, pH 7.4 (PBS). Serial sections were overlaid with 25 μl of a 1:500 dilution of ascitic fluid containing the specific antibodies, anti-T1, anti-T3, anti-T4, anti-T5, anti-T6, anti-T8, and anti-I1 for 60 min. Control sections were incubated with PBS or with control ascitic fluid. All sections were subsequently incubated with rabbit anti-mouse Ig serum (N. L. Cappel Laboratories Inc., Cochranville, Pa.), diluted 1:40, swine anti-rabbit Ig antiserum (Dakopatts A/S), diluted 1:40 and peroxidase rabbit antiperoxidase reagent (PAP) (Dakopatts A/S), diluted 1:50, for 20-30 min. The rabbit anti-mouse Ig antiserum was extensively absorbed with human serum-coated Sepharose 4B beads, liver, and kidney powder before use. Some sections were incubated first with rabbit anti-human IgM serum at a dilution of 1:200, followed by incubations with swine anti-rabbit Ig antiserum and PAP reagent as described above. Between incubations the sections were washed for 10 min in three changes of PBS.

The sections were stained with 3-amino-9-ethylcarbazol (Aldrich Chemical Co., Inc., Milwaukee, Wis.) and H_2O_2 in a 0.1 M acetate buffer, pH 4.9, for 2-3 min to develop the red

¹ Abbreviations used in this paper: anti-I1, anti-Ia monoclonal antibody; PAP, peroxidase-antiperoxidase; PBS, phosphate-buffered saline.

TABLE II
Distribution of T Cell Subsets in Lymph Node Tissue*

Antibody	Secondary follicle				Paracortex	Medulla
	Primary follicles	Mantle zone	Germinal center			
Anti-IgM	Membrane staining of a majority of small lymphocytes	Membrane staining of a majority of small lymphocytes	Intercellular strong staining; weak membrane staining of large cells; some intracytoplasmic staining	Variable, but usually few stained cells	Variable, but usually few stained cells	
Anti-I1	Membrane staining of a majority of small lymphocytes	Membrane staining of a majority of small lymphocytes	No intercellular staining; weak membrane staining of large cells	No staining of a majority of small lymphocytes, intense staining of large cells with cytoplasmic extensions	Variable, but about one-half of the cells stained	
Anti-T1, T3	Membrane staining of few scattered cells	Few scattered stained cells	Crescentic rim of stained cells on border with mantle zone; scattered stained cells inside germinal center	Majority of small lymphocytes stained	Variable, up to one-half of the cells stained	
Anti-T6	No staining	No staining	No staining	No staining of lymphocytes; however, in three lymph nodes a number of large cells showed cytoplasmic staining	No staining	
Anti-T4	Membrane staining of few scattered cells†	Weak staining of few scattered cells	Similar to T1, T3	Majority of small lymphocytes stained but slightly fewer than T1, T3	Variable, up to about one-half of the cells stained	
Anti-T5	Membrane staining of very few scattered cells	Very few scattered stained cells	Generally no stained cells	Minority of small lymphocytes stained	Variable, usually minority of cells stained	
Anti-T8	Membrane staining of a few scattered cells† (more than T5)	Few scattered stained cells (more than T5)	Very few stained cells	Minority of small lymphocytes stained (more than T5)	Variable, usually a minority of cells stained; also some inside sinuses	

* Staining of 4- μ m thick acetone-fixed frozen sections, performed by the PAP technique (see Materials and Methods).

† Intensity of staining was weak with anti-T4 antibody and most intense with anti-T8 antibody.

reaction product (17). The reaction was terminated by washing in acetate buffer. The sections were air-dried and cover slipped with Elvanol (E. I. Du Pont de Nemours & Co., Wilmington, Del.). Photographs were taken with a Zeiss photomicroscope (Carl Zeiss, Inc., New York), employing a green filter.

Results

Control sections that were incubated with PBS in place of specific antibodies showed only nonspecific staining of mast cells, eosinophils, and neutrophils. Sections incubated with control ascitic fluid showed in addition weak granular staining of material that appeared to be intercellular. In contrast, the staining patterns obtained with the specific antibodies were distinctive, as summarized in Table II.

The anti-IgM antiserum resulted in a peripheral pattern of staining, compatible with membrane staining, of most of the small lymphocytes in primary follicles and in the mantle zones of secondary follicles (follicles with germinal centers) (Fig. 1). Moreover, the mantle zones of secondary follicles usually contained a broad rim of positive cells on the capsular side, whereas only a few cells were seen on the medullary side. Most of the large cells in germinal centers showed membrane and/or cytoplasmic staining; in addition, there was some intercellular staining. In the interfollicular areas variable, but usually low numbers of small lymphocytes with membrane staining were found, although in the medulla usually only a few cells with surface IgM were present.

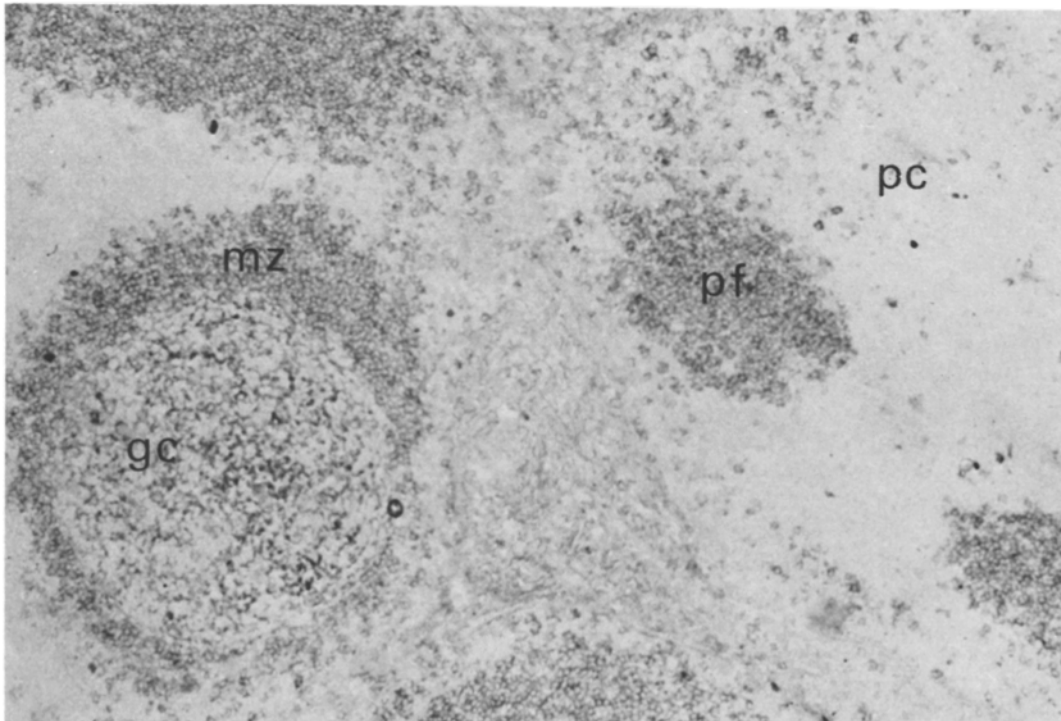


FIG. 1. Lymph node stained for IgM. Peripheral staining of small lymphocytes can be seen in several primary follicles (pf) and in mantle zone (mz) of a secondary follicle. In the germinal center (gc), strong intercellular staining almost obscures weak peripheral staining of large lymphocytes. In the paracortical (pc) area, only a few positive lymphocytes are present. $\times 140$.

The anti-Ia antibody resulted in membrane staining of a majority of lymphocytes in both primary and secondary follicles. The intensity of staining varied, with the small lymphocytes of the mantle zone showing more intense staining than the large germinal center cells. In the paracortical areas, most of the small lymphocytes were not stained; however, scattered between the unstained paracortical lymphocytes were large cells showing diffuse, cytoplasmic staining in an irregular dendritic pattern (Fig. 2). In addition, groups of adjacent small lymphocytes appeared to be surrounded by a ring of staining. Some macrophages, including epithelioid cells, stained diffusely for the Ia antigen.

The two monoclonal antibodies reactive with all peripheral T cells, anti-T1 and anti-T3, produced patterns that were similar to one another, with membrane staining of the majority of small lymphocytes in the paracortical areas and medulla (Fig. 3). Few or no reactive lymphocytes were found within primary follicles; however, there was a large number of positive cells in secondary follicles on the capsular side of the germinal center, which sometimes showed a striking crescentic accumulation (Fig. 3).

Anti-T6 antibody, which defines a major immature thymocyte population, was unreactive with lymphocytes within the lymph node. However, in three lymph nodes (cases 1, 3, and 4), there were scattered large cells with irregular extensions in paracortical areas that showed diffuse cytoplasmic staining with anti-T6 antibody.

The distribution of T cell subsets within the lymph nodes was studied with subset-

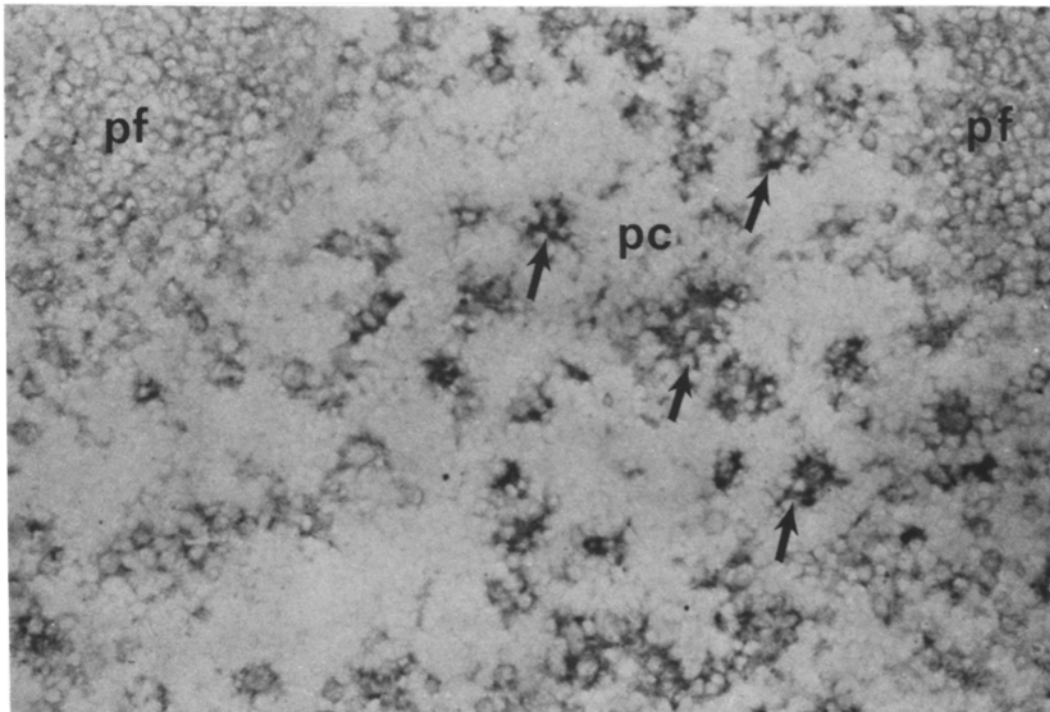


FIG. 2. Lymph node section stained for Ia antigen. Lymphocytes in the follicles (pf) show peripheral staining. The paracortical area (pc) contains mainly negative small lymphocytes with several strongly positive large cells with long cytoplasmic extensions (arrows), sometimes surrounding lymphocytes. $\times 350$.

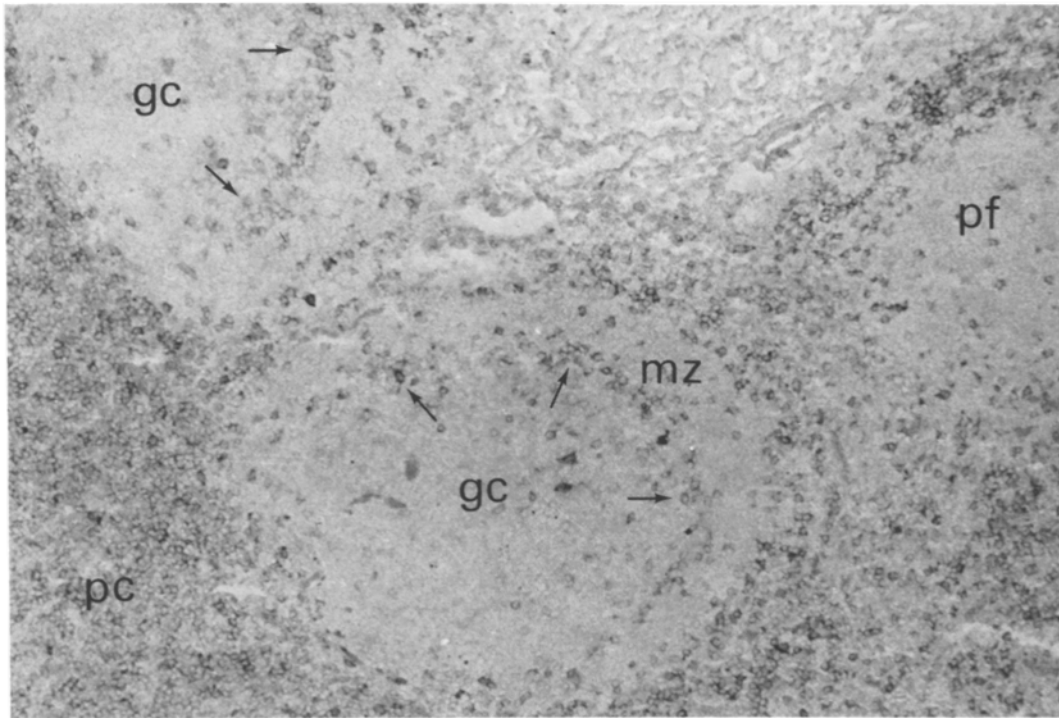


FIG. 3. Lymph node stained with anti-T1 antibody. The majority of cells in the paracortical (pc) areas show peripheral staining. The primary follicle (pf) and germinal centers (gc) contain scattered positive cells. In each secondary follicle a group of positive cells (arrows) can be seen at the periphery of the germinal center (gc). $\times 140$.

specific antibodies. The anti-T4 antibody generally resulted in relatively less intense staining than that defined by the other antibodies; however, it was clear that a majority of small lymphocytes in the paracortical region were reactive (Fig. 4). Moreover, in secondary follicles, anti-T4 antibodies produced the same crescentic shaped rim of reactive cells as was found with anti-T1 and anti-T3 antibodies. The medulla contained numerous positive cells, which were similar in number and distribution to those seen with anti-T1 and anti-T3 antibodies. In addition, some intercellular staining was present in the germinal centers.

The staining seen with anti-T8 antibodies was more intense than with any of the other monoclonal antibodies, and the number of $T8^+$ cells was slightly greater than the number of $T5^+$ cells. Both anti-T5 and anti-T8 antibodies stained only a minority of cells in the paracortical areas (Fig. 5). In primary follicles and in the mantle zone of secondary follicles, only a few scattered $T5^+$ and $T8^+$ cells were observed (Fig. 5). In addition, most germinal centers were devoid of $T5^+/T8^+$ cells, although some contained an occasional stained cell. The medulla contained a minority of $T5^+/T8^+$ cells.

There were differences in the percentages of $T4^+$ cells (inducer/helper) and $T5^+/T8^+$ cells (suppressor/cytotoxic) in the five different lymph nodes examined. In one of the cases of follicular hyperplasia (case 3), a relatively high percentage of $T5^+/T8^+$ cells were found in paracortical areas when compared with the node with follicular

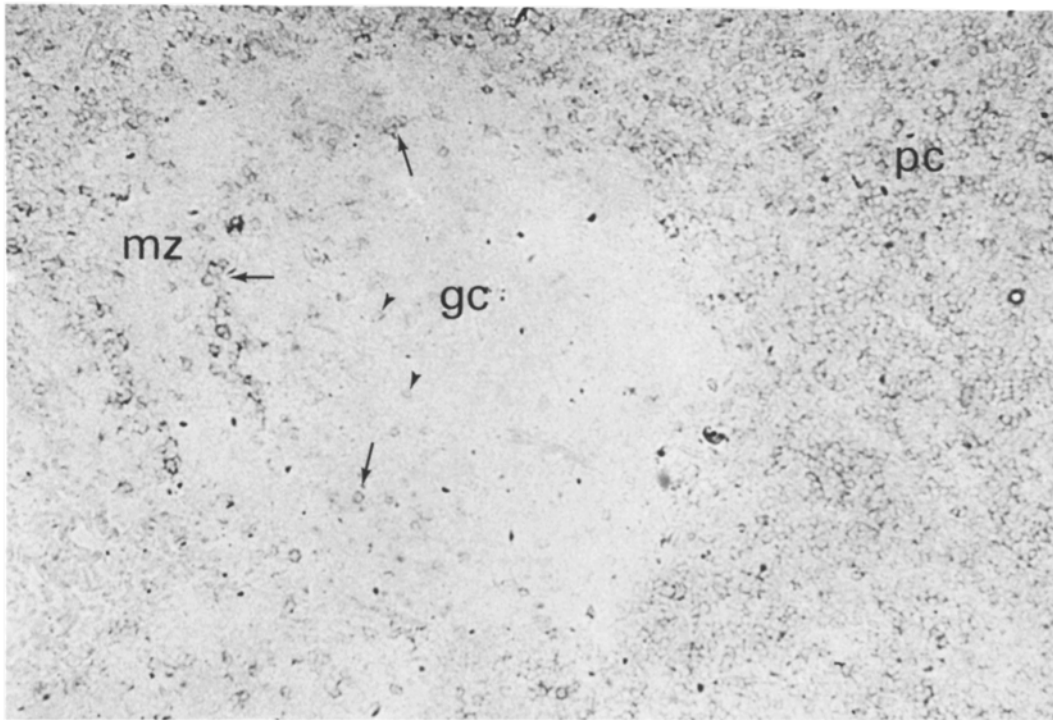


FIG. 4. A portion of a lymph node containing a secondary follicle stained with anti-T4 antibody is shown. The majority of cells in the paracortical area (pc) are stained. Stained cells are also present in the germinal center (gc), either as scattered cells (arrowheads) or arranged in clusters (arrows) at the periphery of the germinal center. $\times 220$.

and diffuse hyperplasia (case 1), whereas the lymph node with sinus histiocytosis from the patient with breast cancer (case 5), showed a low number of $T5^+/T8^+$ cells.

None of the anti-T cell antibodies studied reacted with cells or structures in lymph nodes other than those described above.

Discussion

Employing a series of monoclonal antibodies directed against T cell antigens and Ia antigens, we have studied the distribution of T cell subsets in frozen tissue sections of human lymph nodes by an immunoperoxidase technique. In the paracortical areas, the majority of lymphocytes stained with anti-T1 and anti-T3, two monoclonal antibodies reactive with all mature peripheral T cells. In addition, some $T1^+$, $T3^+$ cells were seen in follicles, especially in the germinal centers. These findings demonstrate the presence of T cells in B cell areas, as has been shown by Gutman and Weissman (5) in lymph nodes of mice through the use of heteroantisera. Furthermore, the majority of $T1^+$, $T3^+$ cells in the paracortex and follicles also reacted with anti-T4 antibody. Thus, the $T1^+$, $T3^+$, $T4^+$ T cell population, which defines the inducer subset, represents the majority of lymph node T cells, as was shown to be the case previously for circulating peripheral blood T lymphocytes (10, 14). In contrast, only a small fraction of cells in the paracortex stained with anti-T5 and anti-T8 antibodies, which identify suppressor/cytotoxic cells.

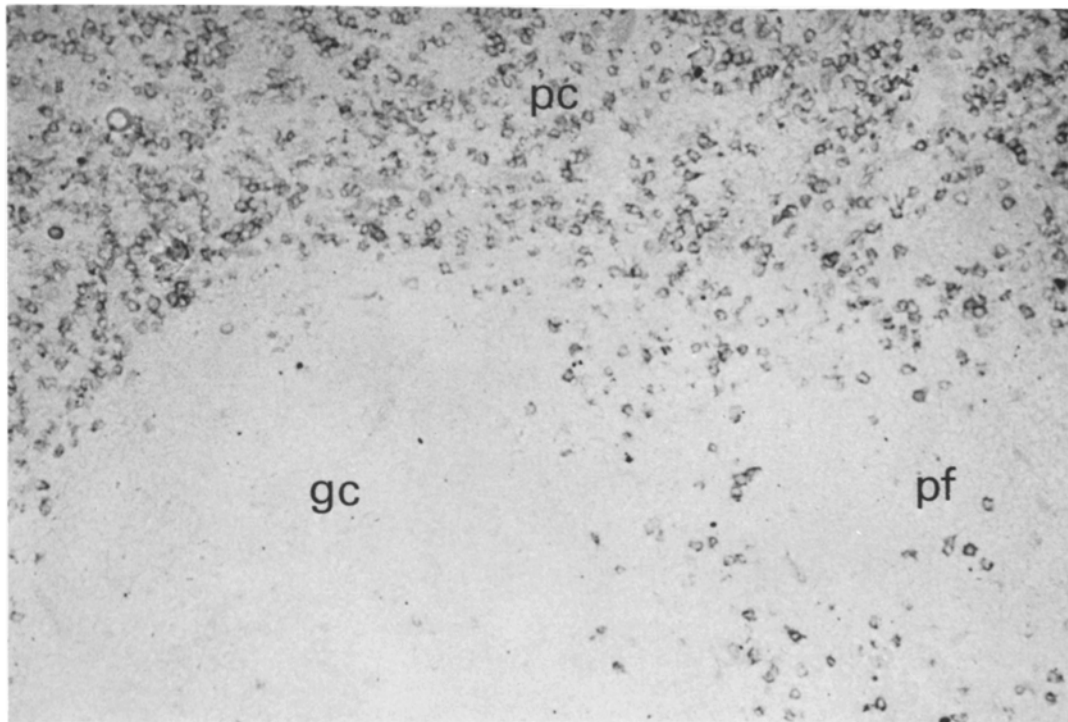


FIG. 5. Lymph node section stained with anti-T8 antibody. Intensely staining lymphocytes are located in the paracortical area (pc). A few scattered positive staining cells are also seen in the primary follicle (pf) but none in the germinal center of a secondary follicle (gc). $\times 220$.

A slightly higher percentage of cells stained with anti-T8 than with anti-T5 antibodies, which indicates that these antibodies did not define precisely the same population of cells. This is in agreement with previous studies of peripheral blood T cell suspensions, which showed 20% of cells to be reactive with anti-T5 and 30% of cells reactive with anti-T8 (12). In studies employing complement-mediated lysis, it was demonstrated that $T4^+$ and $T8^+$ subsets were mutually exclusive, and that the $T8^+$ population contained the entire $T5^+$ subset as well as a $T5^-$, $T8^+$ subset (12). The function of the latter population has not yet been determined.

The most striking result of our study was the finding of large numbers of $T1^+$, $T3^+$, $T4^+$ inducer cells in germinal centers, especially on the capsular side (Figs. 3 and 4, Table I). It is reasonable to assume that their presence reflects a role of T inducer/helper cells in the formation of germinal centers, especially in view of the absence or paucity of $T4^+$ cells in primary follicles. This possibility is supported by several prior observations. Nude mice, which lack germinal centers, develop these structures within 3 wk after thymus grafting (18) or thymus cell injection (19). Furthermore, adult-thymectomized and irradiated rats need T lymphocytes in addition to B cells and antigenic stimulation to restore germinal center formation (20). Patients with congenital thymic aplasia, who are deficient in $T5^+$ cells but possess $T4^+$ cells (21), have germinal centers (22).

It has been shown that antigens draining into regional lymph nodes enter follicles from the subcapsular sinus (23). Therefore, the finding that $T4^+$ cells are found in a

crescentic pattern at the border of mantle zone and germinal center facing the capsular sinus is of interest. This arrangement could allow for direct contact between B lymphocytes in the mantle zone with antigen-primed T helper cells. In contrast, T5⁺/T8⁺ cells, which cannot provide help to B cells (24), are rarely found in germinal centers.

The anti-T6 antibody, which reacts with 70% of thymocytes and not with mature peripheral T cells, did not stain any lymphocytes in the lymph nodes. This finding is consistent with the view that only mature T cells are present in lymph node structures. However, in the paracortex of three lymph nodes, groups of large cells with irregular cytoplasmic extensions showed intense cytoplasmic staining. Anti-T6 antibody stains Ia-positive cells in the epidermis (data not shown), which appear to be Langerhans cells. It, therefore, appears likely that the T6⁺ cells in lymph nodes represent Langerhans cells that have migrated to the regional lymph node from the skin (25). In support of this, one of the lymph nodes (case 1) in which a large number of stained cells were detected showed changes consistent with dermatopathic lymphadenitis.

The anti-Ia antibody, which reacts with a common framework portion of the Ia antigen, stained a large majority of cells in both primary and secondary follicles, which were shown to be B lymphocytes by virtue of their reactivity with IgM antibodies. In addition, although the small lymphocytes in paracortical areas reactive with anti-T1 and anti-T3 antibodies were not stained, scattered large cells with irregular extensions showed diffuse intense staining of the cytoplasm. The morphological appearance and location of these cells correspond with those of interdigitating reticulum cells (26).

Interdigitating reticulum cells, which have been described in several species, including rabbit (26), rat (27), and man (28), appear to be present exclusively in T-dependent areas. The staining pattern of these cells, with extensions in between and surrounding small lymphocytes, is similar to that of Ia-positive cells in the thymic cortex, which have been interpreted as thymic epithelial and/or reticulum cells (15, 29). The presence of relatively large amounts of Ia antigen on interdigitating cells supports the concept that these cells may play a role in the presentation of antigen to T lymphocytes. Ia-positive, antigen-presenting cells have been demonstrated in peritoneal fluid (30, 31), spleen cell suspensions (32), and skin (Langerhans cells) (33).

In summary, the present findings support the concept that T cells and B cells are generally located within separate compartments in human lymph nodes. Thus, T cells are largely restricted to paracortical areas and B cells to the follicles. Moreover, the majority of T cells in paracortical areas are of the T1⁺, T3⁺, T4⁺ (inducer/helper) subset. In addition, a small number of T1⁺, T3⁺, T4⁺ cells are located in the germinal centers of follicles, within the mantle zone. This population, which is known to have inductive properties (10), is strategically located within lymph nodes to participate in B cell differentiation and maturation.

Summary

A series of T cell-specific monoclonal antibodies was used to determine the location of T lymphocyte subpopulations in frozen sections of human lymph nodes by means of an immunoperoxidase technique. The majority of cells in the paracortical regions were reactive with anti-T1 and anti-T3 antibodies, which define all mature peripheral T cells. In contrast, the majority of cells within primary follicles were unreactive with

anti-T1 and anti-T3 antibodies, but were reactive with anti-Ia and anti-IgM antibodies. In addition, a substantial number of T1⁺, T3⁺ cells were found in the germinal centers of secondary follicles on the capsular side. The vast majority of T1⁺, T3⁺ cells in the paracortex and the follicles were reactive with anti-T4 antibody, which defines inducer/helper T cells. Only a minority of cells in these areas were reactive with anti-T5 and anti-T8 antibodies, which define cytotoxic/suppressor cells. No lymphocytes were stained with anti-T6 antibody, which reacts with a majority of thymocytes but not with peripheral T cells. Scattered cells in the paracortex showed staining for Ia antigen in an irregular dendritic pattern. The findings demonstrate that the major T cell population found within human lymph node bears the mature T1⁺, T3⁺, T4⁺ phenotype characteristic of inducer T cells. Moreover, the location of this population indicates that they play a role in the induction of B cell differentiation in vivo.

The authors wish to thank Ms. Diane Crawford and Ms. Patricia Della Pelle for excellent technical assistance and Ms. Michele Angelo for the skillful typing of this manuscript.

Received for publication 4 August 1980 and in revised form 24 September 1980.

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